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# Inheritance Studies of the Reaction of Selfed Lines of Maize to Smut (*Ustilago zeae*)

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THE COMMON OR BOIL SMUT, *Ustilago zeae*, has nearly a world-wide distribution and is easily recognized by characteristic smut masses or tumors which may appear on any of the aerial parts of the plant. It is a curious fact that although corn is native to America and smut undoubtedly has occurred here for centuries, the disease was first described by the French botanists, Bonnet *(13)*† in 1754, and Aymen in 1760. Schweinitz in 1822 was the first to describe the disease in the United States.

Up to about the middle of the 19th century corn smut was believed to be a physiological disturbance due to "too great an abundance of sap which in rich land is carried towards certain portions of the plant" (Tillet). This concept was destroyed by the discovery of the parasitic nature of the fungus by Kuehn, Brefeld, and others who established the fact that this fungus was not seed-borne, as is the case with most of the cereal smuts, but that infections are local and can occur in any actively growing tissues. It remained for Stakman and his co-workers to point out the presence of physiologic forms of smut and demonstrate that the pathogene is predominantly heterothallic, the fusion of lines of opposite sex being prerequisite to infection.

The mature smut tumor consists of a thin membrane derived from host tissue which at first is gray but later becomes dark in color. The membrane encloses a mass of spores (chlamydospores), fibrovascular bundles, and parenchyma cells of the host plant. These

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†Reference by number is to "Literature Cited," pages 31 and 32.

spores are set free by the rupture of the membrane and may be carried by wind, on refuse, or by other agencies to suitable locations, where they germinate.

The mature chlamydospores are diploid. On germination they normally produce a pro-mycelium in which reduction division apparently occurs. The sporidia are uninucleate and grow by budding in a typically yeast-like fashion; thus the progeny of a single sporidium should constitute a clonal line. This habit of growth and relative ease with which pure sporidial lines can be isolated makes smut a very desirable material for the study of mutations.

Stakman *(33, 34, 35)* and his co-workers have demonstrated that most monosporidial lines alone cannot cause normal infection in the host. It therefore becomes necessary to test a combination of forms. Culture media have not proved satisfactory in this work for, although monosporidial lines will grow profusely in culture, it appears that diplonts can be produced only in the living host.

In recent studies on rate of mutation of smut from different geographical locations, Stakman has shown that the mutation rate is extremely high in certain monosporidial lines. This is of particular interest since West Virginia strain A-8 was found to be one of the most prolific, giving 162 distinct mutants during the course of one year. Because of this rapid mutation rate and the resulting hybridization that might follow, the number of physiologic forms present in the West Virginia corn nursery must be exceedingly high. There seems to be little doubt about the presence of physiologic forms and the hybridization of these forms under natural conditions. Any improvement of corn by breeding methods should take into account the fact that physiologic strains do exist and that the probability is that new forms may arise from time to time.

The annual loss due to smut in the United States is variable and rather difficult to measure, but an estimate of between two and three percent by the Plant Disease Reporter *(29)* of the United States Department of Agriculture is probably as good a figure as is available at the present time. Under certain conditions this loss may be much higher, and figures ranging from five to twenty percent are frequently reported for some states. Careful re-survey work carried on at the Minnesota Experiment Station *(18)* indicates that the figures given by the Plant Disease Reporter are somewhat lower than the actual field conditions indicate. This loss due to smut is most apparent in areas where corn is grown for market, since the presence of smut makes the ear unfit for canning or table consumption.

There is no close agreement among investigators as to the effect of environmental factors on the prevalence of smut. Many of the earlier workers, among them Hitchcock and Norton *(14)* as well as Selby and Hickman *(32)*, felt that any conditions which stimulate the host to vigorous growth or increased succulence seem to increase the chances for infection. Coffman, Tisdale, and Brandon *(4)*

making observations at Akron, Colorado, state that smut is most prevalent during dry seasons. This is thought to be due in part to better survival and dissemination of chlamydospores as well as to the higher temperatures that occur during dry seasons. Immer and Christensen *(18)* at Minnesota also found that dry weather conditions tend to give a heavy smut infection. Potter and Melchers *(30)* in reporting studies on the ecological relations of smut state that the stage of development of the host is more important than the time of season. Leaf, tassel, shoot, and basal nodes or suckers represent the stages in the development of a plant when meristematic activity is at a maximum and infection is most likely to occur. Moisture is not a limiting factor for the fungus so long as there is sufficient for host development. Tisdale and Johnston *(36)* working with seedlings in the greenhouse were able to get a heavy infection only when humidity was high and a temperature of 80° - 95° F. was maintained.

Considerable data have been accumulated by different investigators as regards the effect of smut on yield. Garber and Hoover *(9)* in working with the $F_1$ hybrids between selfed strains found that barrenness was greatly increased among the smutted plants, but that if a plant, although infected with smut, is able to produce an ear, the yield from such a plant was not materially less than from adjacent non-infected plants. These observations were more marked when groupings of the smutted plants were made according to the region of the plant where infection occurred. Plants with tassel infection produced significantly greater yields of shelled corn than uninfected plants within the same strain. Recent work by Kyle *(24)*, where studies of the relation between vigor of the corn plant and its susceptibility to smut were reported, indicate that the more vigorous plants in general are those that have the highest percentage of smut. Kyle points out the danger in the procedure of most plant breeders who select within their selfed lines of corn only those strains that are smut free. This selection he feels will in time tend to eliminate not only the smut-susceptible strains but also those strains that are potentially high in yield. On the other hand Immer and Christensen *(16)* at Minnesota, as well as Jorgensen *(22)* at Ohio, found that smut tended to decrease the yield and that smut infections located above the ear tend to reduce the yield significantly more than did galls below the ear.

Many plant breeders have been able to isolate selfed lines of maize that show differential susceptibility to smut. The behavior of certain crosses between high and low susceptible lines is such as to show that resistance or susceptibility represents strain characteristics. At the time the present work was undertaken, the writer was not aware of the inheritance studies that were being conducted by Dr. Immer *(17)* at Minnesota. This investigation parallels the work of Dr. Immer rather closely in certain phases and it is interesting to compare the similarity of conclusions regarding the genetic

aspects of smut inheritance. The following report is an attempt to demonstrate the inheritance of smut reaction in crosses between certain selfed strains and to show the relation between smut reaction and certain known heritable characters.

## MATERIALS AND METHODS

The data reported in this paper were obtained from studies of the smut reaction of certain selfed lines of maize that were being carried as a part of the general corn breeding project at the West Virginia Agricultural Experiment Station. During the past ten years more than 200 selfed strains of maize have been grown under smut-epiphytotic conditions and careful notes recorded for the smut reaction of each plant during the season. The general plan has been to grow each strain in duplicated single row plats, each plat consisting of 50 single plant hills spaced 18 inches apart in the row. The smut epiphytotic was created by scattering smut-contaminated horse manure three times during the growing season. The first application was made when the plants were approximately knee high; this treatment was followed at two-week intervals by the second and third applications.

It was found convenient to take the smut notes on mimeographed sheets, each one of which was ruled and numbered from one to fifty, and divided into columns to indicate the place of appearance of the smut boil on the plant, such as tassel, neck (region between tassel and top leaf of plant), leaf, ear, below ear, and base. Sizes of smut boil were indicated by the following numeral grades: No. 1 (size of grain of wheat), No. 2 (size of marble), No. 3 (size of walnut), and No. 4 (larger than walnut). Three men worked together in taking the smut notes. One individual acted as recorder while the other two (one on each side of the row) carefully examined the plants for smut. In this manner each plant was carefully examined, and the amount and place of smut recorded. Smut data were usually taken at three dates: the first shortly after the plants had finished silking, the second some three weeks later but before the leaves had begun to shatter, and the third and last, at the time of husking. Attention was given chiefly to the prevalence of ear smut at the third time.

Seed from a strain of corn known to be rather highly susceptible was planted in every tenth row throughout the nursery to serve as a check upon the extent and uniformity of the smut epiphytotic produced. The amount of smut varied somewhat from year to year but there was no year in which a "satisfactory" smut epiphytotic failed to appear. The range of infection of the susceptible checks was found to be from 40 to 80 percent, with an average of 62 percent smutted plants for a nine-year period. Selection was practiced among the selfed lines of corn with the hope of establishing certain lines that were immune to smut.

## SMUT REACTION OF CERTAIN SELFED LINES OF MAIZE, AND CROSSES BETWEEN THESE SELFED LINES

The results of the smut reaction of certain selfed lines of maize over a period of years are given in Table 1. It will be noticed that selfed lines differ in regard to the evident amount of smut infection so that it would be a relatively easy matter to isolate high-infection and low-infection selfed lines. There is also a marked tendency for certain lines to show susceptibility in a particular part of the plant. Thus one can identify tassel and base strains as indicated in Table 1.

The inheritance studies deal with crosses among these West Virginia strains as well as crosses between these strains and genetic linkage testers of unknown smut reaction. It is in order here to point out that the tassel strain has shown fairly high susceptibility and that over 80 percent of the smut observed in these selfed plants is located in the tassel.

The base strain is highly susceptible. On the average, over 75 percent of all plants were smutted, and over 80 percent of all the

TABLE 1—*Smut reaction of certain selfed lines of maize with number of plants, place, and percentage of smut infection*

| Name of strain | Year | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent |
|---|---|---|---|---|---|---|---|---|---|
| Tassel | 1924 | 23 | 8 | .. | .. | .. | .. | 1 | 39.1 |
| | 1925 | 87 | 14 | .. | .. | .. | .. | 3 | 19.5 |
| | 1926 | 94 | 38 | .. | .. | .. | 2 | .. | 42.6 |
| | 1927 | 86 | 27 | .. | .. | .. | .. | .. | 31.4 |
| | 1928 | 68 | 22 | .. | 1 | 4 | 1 | .. | 41.2 |
| | 1929 | 146 | 91 | 1 | .. | 6 | 4 | .. | 69.9 |
| | 1930 | 172 | 17 | .. | 1 | 1 | 9 | .. | 16.3 |
| | 1931 | 188 | 110 | 1 | 7 | 10 | 7 | 8 | 76.1 |
| | Total | 864 | 83.0 | 0.5 | 2.3 | 5.3 | 5.8 | 3.0 | 45.6 ave. |
| Base | 1926 | 50 | .. | .. | 5 | 5 | 13 | 6 | 58.0 |
| | 1929 | 49 | .. | 7 | 2 | 6 | 4 | 7 | 53.1 |
| | 1930 | 102 | .. | .. | .. | 7 | 62 | 6 | 73.5 |
| | 1931 | 142 | 6 | 1 | .. | 1 | 87 | 35 | 91.5 |
| | Total | 343 | 2.3 | 3.1 | 2.7 | 7.3 | 63.8 | 20.8 | 75.8 ave. |
| Yellow-resistant | 1922 | 52 | .. | .. | .. | .. | .. | .. | 0.0 |
| | 1923 | 23 | 1 | .. | .. | .. | .. | .. | 4.3 |
| | 1924 | 24 | .. | .. | .. | .. | .. | .. | 0.0 |
| | 1925 | 91 | .. | .. | .. | .. | .. | .. | 0.0 |
| | 1926 | 94 | .. | .. | 1 | .. | .. | .. | 1.1 |
| | 1927 | 96 | .. | .. | .. | .. | .. | 1 | 1.0 |
| | 1928 | 94 | .. | .. | .. | 1 | .. | 1 | 2.1 |
| | 1929 | 149 | .. | .. | .. | .. | 1 | .. | 0.7 |
| | 1930 | 177 | .. | 1 | .. | .. | 2 | 2 | 2.8 |
| | 1931 | 131 | 6 | .. | .. | 1 | .. | 1 | 6.1 |
| | Total | 931 | .. | .. | .. | .. | .. | .. | 2.0 ave. |
| White-resistant | 1922 | 49 | 2 | .. | .. | .. | .. | .. | 4.1 |
| | 1923 | 21 | .. | .. | .. | .. | .. | .. | 0.0 |
| | 1924 | 19 | .. | .. | .. | .. | .. | .. | 0.0 |
| | 1925 | 80 | 2 | .. | .. | .. | .. | .. | 2.5 |
| | 1926 | 96 | .. | .. | .. | .. | 1 | .. | 1.0 |
| | 1927 | 97 | 1 | .. | .. | .. | .. | .. | 1.0 |
| | 1928 | 94 | 3 | 1 | .. | 2 | .. | .. | 6.4 |
| | 1929 | 142 | .. | .. | .. | 1 | .. | .. | 0.7 |
| | 1930 | 162 | .. | 1 | .. | .. | .. | .. | 0.6 |
| | 1931 | 200 | 7 | .. | 1 | 1 | .. | .. | 4.5 |
| | Total | 960 | .. | .. | .. | .. | .. | .. | 2.4 ave. |

smutted plants were smutted in the lower region of the plant. Base and below-ear infection are considered together in the discussion of smut reaction.

Table 1 does not take into account the secondary infections that may be present in a given plant, but classifies a plant in the column which represents the position of the largest smut boil. Table 2 has been constructed to show these secondary smut infections and reveals a very distinct difference between the tassel and base strains which is not evident from a study of Table 1 alone. For example, Table 1 classifies 67 smutted plants in categories other than tassel; Table 2 shows that 29 of these 67 (or 43 percent) are also infected in the tassel. This further emphasizes the fact that this strain has a pronounced predisposition for smut susceptibility in the tassel. The places of secondary infection in the base strain show a more general distribution. Of the 220 plants that fall in the base and the below-ear primary classes, only 69 (31.4 percent) also show secondary infection in this same region, while 33 plants (15 percent) show secondary infections in the tassel. In considering the crosses between these strains it may be well to have this smut relation of the parents in mind when an interpretation of the observed data is attempted.

TABLE 2—*Multiple smut infection for all plants of the tassel and base strains*

| | Primary infection | | Secondary infection | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Place and number of plants | | | | | |
| | Place | No. of plants | Tassel | Neck | Leaf | Ear | Below ear | Base |
| Tassel strain | Tassel | 327 | 0 | 1 | 2 | 0 | 1 | 0 |
| | Neck | 2 | 1 | 0 | 0 | 0 | 0 | 0 |
| | Leaf | 9 | 2 | 0 | 0 | 0 | 1 | 0 |
| | Ear | 21 | 14 | 0 | 0 | 0 | 1 | 0 |
| | Below ear | 23 | 8 | 0 | 0 | 0 | 0 | 0 |
| | Base | 12 | 4 | 0 | 0 | 0 | 0 | 0 |
| Base strain | Tassel | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Neck | 8 | 0 | 0 | 1 | 0 | 1 | 1 |
| | Leaf | 7 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Ear | 19 | 1 | 1 | 1 | 0 | 4 | 3 |
| | Below ear | 166 | 28 | 5 | 14 | 6 | 0 | 66 |
| | Base | 54 | 5 | 4 | 8 | 1 | 3 | 0 |

The yellow and white-resistant strains show a marked resistance to smut when their reaction over a period of years is studied. These plants did not show any secondary infection and are therefore not presented in such a table. The smutted plants that were observed in the yellow-resistant strain show rather general distribution of the place of infection. In the white-resistant strain, which shows about the same average infection as the yellow-resistant, there seems to be a tendency for infection in the smutted plants to be limited to the tassel since 15 out of a total of 23 plants (or 65 percent) show tassel infection.

Table 3 shows the smut reaction of the $F_1$, $F_2$, and backcross plants obtained from crosses between West Virginia tassel, base,

yellow-resistant, and white-resistant selfed strains. The original crosses were made in 1929 and the $F_1$ plants were grown in 1930. Certain of these $F_1$ plants were backcrossed to their respective parents while others were self-pollinated. The backcross or the $F_2$ plants were grown in 1931 and furnish the bulk of the data in Table 3. Wherever possible, data were obtained from backcross in preference to $F_2$ plants since it was felt that fewer plants would be required to give the necessary data for genic analysis.

**Table 3**—*Smut reaction of the $F_1$, $F_2$, and backcross plants from crosses between certain West Virginia selfed lines*

| Description of parents | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|
| (Tassel x base) $F_1$ | 101 | 5 | 0 | 3 | 2 | 14 | 5 | 28.7 | |
| (Base x tassel) $F_1$ | 113 | 10 | 0 | 1 | 0 | 29 | 7 | 41.6 | 4.12 |
| (Tassel x base) $F_2$ | 93 | 27 | 1 | 3 | 5 | 9 | 2 | 50.5 | |
| (Base x tassel) $F_2$ | 41 | 15 | 4 | 0 | 0 | 0 | 2 | 51.2 | 0.13 |
| (Tassel x base) x base | 56 | 0 | 1 | 5 | 3 | 25 | 11 | 80.4 | |
| (Base x tassel) x base | 92 | 0 | 1 | 15 | 2 | 45 | 6 | 75.0 | 1.51 |
| (Base x tassel) x tassel | 18 | 0 | 0 | 2 | 0 | 3 | 0 | 83.3 | |
| (Tassel x yel.-res.) $F_1$ | 125 | 17 | 0 | 2 | 1 | 7 | 1 | 22.4 | |
| (Yel.-res. x tassel) $F_1$ | 45 | 6 | 0 | 0 | 0 | 5 | 4 | 33.3 | 2.3 |
| (Tassel x yel.-res.) $F_2$ | 67 | 26 | 0 | 0 | 0 | 7 | 0 | 49.2 | |
| (Tassel x yel.-res.) x tassel | 5 | 4 | 0 | 0 | 0 | 0 | 0 | ... | |
| (Yel.-res. x tassel) x tassel | 79 | 27 | 0 | 0 | 0 | 8 | 4 | 49.4 | |
| (Tassel x yel.-res.) x yel.-res. | 60 | 9 | 1 | 2 | 0 | 4 | 4 | 33.3 | |
| (Yel.-res. x tassel) x yel.-res. | 84 | 5 | 2 | 1 | 0 | 6 | 3 | 20.2 | 3.2 |
| (Tassel x white-res.) $F_1$ | 120 | 12 | 0 | 3 | 1 | 3 | 0 | 15.8 | |
| (Wh.-res. x tassel) $F_1$ | 66 | 4 | 0 | 0 | 0 | 4 | 1 | 13.5 | 0.55 |
| (Tassel x wh.-res.) $F_2$ | 75 | 24 | 3 | 2 | 2 | 2 | 1 | 45.3 | |
| (Wh.-res. x tassel) $F_2$ | 100 | 5 | 1 | 1 | 2 | 3 | 1 | 13.0 | 8.32 |
| (Tassel x wh.-res.) x wh.-res. | 46 | 8 | 0 | 0 | 0 | 0 | 0 | 17.4 | |
| (Wh.-res. x tassel) x wh.-res. | 98 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 4.35 |
| (Base x yel.-res.) $F_1$ | 120 | 0 | 2 | 1 | 0 | 16 | 3 | 18.3 | |
| (Yel.-res. x base) $F_1$ | 111 | 0 | 5 | 2 | 1 | 17 | 5 | 27.0 | 3.06 |
| (Base x yel.-res.) x base | 93 | 0 | 0 | 3 | 0 | 54 | 13 | 75.3 | |
| (Yel.-res. x base) x base | 78 | 5 | 2 | 3 | 0 | 28 | 18 | 71.8 | 1.02 |
| (Base x yel.-res.) x yel.-res. | 94 | 7 | 0 | 0 | 1 | 5 | 3 | 17.0 | |
| (Yel.-res. x base) x yel.-res. | 97 | 4 | 6 | 2 | 5 | 3 | 0 | 20.6 | .92 |
| (Base x wh.-res.) $F_1$ | 55 | 2 | 0 | 0 | 0 | 1 | 0 | 5.4 | |
| (Wh.-res. x base) $F_1$ | 68 | 1 | 1 | 2 | 1 | 3 | 0 | 11.7 | 3.06 |
| (Wh.-res. x base) $F_2$ | 96 | 2 | 6 | 7 | 1 | 9 | 2 | 28.1 | |
| (Base x wh.-res.) x base | 96 | 6 | 2 | 2 | 1 | 24 | 11 | 47.9 | |
| (Base x wh.-res.) x wh.-res. | 109 | 7 | 1 | 1 | 0 | 1 | 0 | 9.2 | |
| (Wh.-res. x base) x wh.-res. | 93 | 3 | 2 | 0 | 0 | 1 | 0 | 6.4 | 1.64 |
| (Yel.-res. x wh.-res.) $F_1$ | 89 | 4 | 1 | 0 | 0 | 0 | 0 | 5.6 | |
| (Wh.-res. x yel.-res.) $F_1$ | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.95 |
| (Yel.-res. x wh.-res.) x yel.-res. | 83 | 1 | 0 | 0 | 0 | 2 | 0 | 3.6 | |
| (Wh.-res. x yel.-res.) x yel.-res. | 87 | 2 | 0 | 1 | 0 | 1 | 1 | 5.7 | 1.29 |
| (Yel.-res. x wh.-res.) x wh.-res. | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| (Wh.-res. x yel.-res.) x wh.-res. | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| (Wh.-res. x yel.-res.) x wh.-res. selfed $F_3$ | 49 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | |
| (Wh.-res. x yel.-res.) x yel.-res. selfed $F_3$ | 53 | 0 | 0 | 0 | 0 | 1 | 0 | 1.9 | 1.51 |

*Tassel x Base*

The $F_1$ plants from the cross (tassel x base) show 28.7 percent smut, with 65.5 percent of the smutted plants infected in the basal region. The smut reaction of the $F_1$ plants from the cross (base x tassel) gave a total of 41.6 percent smut, with 76.6 percent of the smutted plants infected in the basal region. The $F_1$ plants were grown during 1930, a very dry season, and the wide differences in total amount of smut observed in the reciprocal crosses may be due in part to the uneven smut epiphytotic that prevailed. The plants from both direct and reciprocal $F_1$ crosses showed a similar behavior in the region of the plant where infection occurred. It is also apparent that the "base" strain shows greater influence on the place of infection in the $F_1$ plants than the "tassel" parent.

The $F_2$ plants from the cross (tassel x base) show 50.5 percent of the plants smutted. This is essentially the same as the smut reaction of the $F_2$ plants from the (base x tassel) cross, which gave 51.2 percent smutted $F_2$ plants. The majority of the $F_1$ plants from these crosses showed basal infection, whereas in the $F_2$ plants from the same crosses most plants are infected in the tassel. The amount of smut was found to be intermediate between that of the two parent strains.

Backcross plants obtained from the cross (tassel x base) x base gave 80.4 percent infected plants, and over 80 percent of the smutted plants showed basal infection. The backcross plants obtained from the cross (base x tassel) x base showed a total of 78.5 percent infection, with 74 percent of the infected plants showing smut boils in the basal region of the plants. It is clearly shown here that the base strain is a highly susceptible one and that, within any genotype, the tendency for basal infection is greatly increased by the recurrent use of this strain.

Backcross plants obtained from the cross (base x tassel) x tassel gave a smut infection of 83.3 percent, with two-thirds of the smutted plants showing tassel infection. This indicates that when the "tassel" strain is used as the recurring parent there is a tendency to increase the tassel infections in the progeny. It is unfortunate that there were so few plants from this cross.

*Tassel x Yellow-Resistant*

The $F_1$ plants obtained from the cross (tassel x yellow-resistant) gave 22.4 percent smut, while the reciprocal $F_1$ (yellow-resistant x tassel) gave 33.3 percent smutted plants. Most of the smutted plants were infected in the tassel. The differences in the amount of smut in the direct and reciprocal $F_1$ are wide but are probably not significant. The small number of plants obtained in the cross (yellow-resistant x tassel) may account for the differences in the observed percentage.

The $F_2$ plants obtained from the cross (tassel x yellow-resistant) gave a total infection of 49.4 percent. Nearly 79 percent of these

smutted plants were infected in the tassel. This, it will be recalled, was also found to be the condition in the $F_2$ plants from the (tassel x base) cross.

The backcross plants obtained from a cross of (yellow-resistant x tassel) x tassel gave a total smut infection of 49.4 percent, with nearly 70 percent of the smutted plants showing infection in the tassel.

The backcross plants obtained from crossing the $F_1$ back on the yellow-resistant parent showed less smut than when the cross was made back to the tassel parent. These results are in keeping with those previously obtained and show that the recurrent parent usually dominates the smut reaction in backcross material.

*Tassel x White-Resistant*

The $F_1$ plants obtained from the cross (tassel x white-resistant) gave 15.8 percent smut. Most of the smutted plants were infected in the tassel. The reciprocal $F_1$ plants obtained from the cross (white-resistant x tassel) gave a smut reaction of 13.5 percent. It will be recalled that over a period of years there was found but a slight difference in the amount of smut observed in the yellow-resistant and white-resistant selfed strains. In crosses involving these two strains, the white-resistant parent seems to show a great deal more resistance than does the yellow-resistant parent. In the case at hand the $F_1$ (tassel x yellow-resistant) gave smut percentages of 22.4 and 33.3 for the direct and reciprocal $F_1$ plants, whereas the comparable crosses (tassel x white-resistant) with the white-resistant parent gave 15.8 percent and 13.5 percent smut infection. This greater efficiency of the white-resistant over the yellow-resistant parent can be observed in every cross where a direct comparison is possible.

$F_2$ plants obtained from the cross (tassel x white-resistant) gave a percent smut infection of 45.3. This is based on a population of 75 plants and represents an exceptionally heavy infection for plants where the white-resistant strain was one of the parents. The $F_2$ plants obtained from the cross (white-resistant x tassel) gave a smut infection of 13.0 percent, which is in keeping with the expected results.

Backcross plants obtained from the cross (tassel x white-resistant) x white-resistant gave a smut infection of 17.4 percent as compared to the reciprocal cross (white-resistant x tassel) x white-resistant with a smut infection of 1 percent. With the one exception noted, the smut reaction of all crosses involving the white-resistant parent have given plants that show rather high resistance to smut.

*Base x Yellow-Resistant*

$F_1$ plants obtained from the cross (base x yellow-resistant) gave a smut infection of 18.3 percent, with over 85 percent of the smutted plants showing basal infection. The $F_1$ plants obtained from the cross (yellow-resistant x base) show a smut infection of 27.0 per-

cent, with over 73 percent of the smut boils appearing in the basal region.

Backcross plants obtained from the cross (base x yellow-resistant) x base show a total of 75.3 percent smut, with over 90 percent of the smutted plants infected in the basal region. The reciprocal cross of (yellow-resistant x base) x base gave a total of 71.8 percent smut, with over 82 percent of the plants with basal infections. These backcross results are similar in amount and place of infection and do not show a difference of statistical significance.

Backcross plants obtained from the cross (base x yellow-resistant) x yellow-resistant gave a smut infection of 17 percent. No tendency was noted for the boils to appear in any particular part of the infected plants. The backcross plants from the cross (yellow-resistant x base) x yellow-resistant gave a smut infection of 20.6 percent. The interesting feature about these results lies in the comparison of the backcrosses. When the recurrent parent was the "yellow-resistant" instead of the "base", the percent smut infection was reduced from 75.3 and 71.8 for the direct and reciprocal crosses to 17.0 and 20.6 percent, respectively. This indicates that the yellow-resistant parent is capable of contributing a comparatively high degree of resistance to its progeny. It is equally apparent that, with the recurrence of the "base" strain in the genotype, the total amount of infection is greatly increased and the likelihood of the occurrence of smut on the basal part of the plant is also greatly increased.

*Base x White-Resistant*

Data obtained from crossing the West Virginia "base" strain x white-resistant are comparable to the same crosses made with the yellow-resistant strain, except that the progeny of white-resistant crosses seems to be even more resistant than that of the yellow-resistant. A comparison of the values of smut reported in the last column of Table 3 for the comparable crosses clearly indicates these differences.

*Yellow-Resistant x White-Resistant*

The observed behavior of the yellow-resistant and white-resistant strains when crossed with tassel selfed line and base selfed line leads one to expect to find but little smut when these strains are themselves intercrossed. This is indeed the case, as the data in Table 3 indicate. Even here, however, it is evident that the white-resistant parent is the one contributing the greater resistance to any given cross. The $F_3$ data indicate the possibility of the isolation of types from the crosses between yellow-resistant and white-resistant strains that are still more highly resistant to smut and that show considerable promise as foundation stock for further breeding work.

From a study of data presented in Tables 1, 2, and 3 it becomes evident that selfed strains differ sharply in regard to amount and

position of smut infection. Although distinct differences are apparent for the different strains, the behavior of any one remains remarkably uniform over a period of years. This would suggest that the basis for the differences observed is due to variations in genotypes and therefore heritable.

From the evidence obtained from strain intercrosses that differ in respect to susceptibility and place of infection, it may be clearly demonstrated that the susceptibility to smut infection is transmitted from parent to offspring. The fact that no distinct observable ratios can be identified in the backcross or the $F_2$ progeny from a given cross is some evidence that several factors play a part and are responsible for the observed differences. There is little doubt that the "resistant" parents as well as the "base" and "tassel" strains are able to transfer their own peculiarities to their offspring; this was particularly evident from a study of backcross data. Furthermore, this resistance or susceptibility is probably physiological, since no morphological differences were apparent which could account for the smut reaction observed in the parents or offspring.

## GENETIC ANALYSIS OF WEST VIRGINIA SELFED STRAINS, AND SMUT REACTION OF CROSSES WITH KNOWN GENETIC TESTERS

The observations reported in Section III led to an attempt to determine, if possible, any linkage relation that might exist between the factor or factors responsible for smut inheritance and other known genetic factors. Accordingly, crosses were made between the different West Virginia selfed lines and linkage testers which carried known genetic factors, to see if evidence for the inheritance of smut reaction could be obtained by observing the inheritance of these known factors in relation to smut infection. With this study in mind genetic material representing nine linkage groups was assembled, and appropriate crosses were made.

West Virginia tassel, base, yellow-resistant, and white-resistant strains were crossed with the appropriate linkage tester and smut notes recorded for the $F_1$ plants as well as for the backcross and $F_2$ segregating progenies. In presenting the data obtained, the results of crossing each linkage tester with the West Virginia strains are given and discussed separately. It should be pointed out here that the linkage testers used were of unknown behavior regarding smut reaction; however, such evidence as has been accumulated during the time these testers have been grown in West Virginia indicates that all of them are rather highly susceptible. This suggests that the most critical evidence on the inheritance of smut reaction should be sought in the progeny from crosses involving the resistant strains.

West Virginia tassel, base, and yellow-resistant strains are all yellow in color, while the white-resistant lacks the yellow endosperm. These strains were isolated from Leaming, Lancaster Sure Crop,

Reid's Yellow Dent, and Boone County White varieties, respectively. As a result of crosses made with the genetic testers used, it was found that all four West Virginia strains lacked dominant C and R of the aleurone series but were normal in regard to all other genetic factors for which tests were made.

*Linkage Group I**

The genetic tester used to test Linkage Group I was known to be of the following genotype: AA CC RR shsh wxwx Prpr. West Virginia tassel, base, yellow-resistant, and white-resistant strains were each pollinated with the shrunken-waxy tester. The crossed seed was characteristic of a purple aleurone over a yellow endosperm, except for the ears resulting from the cross with white-resistant, which gave seed light purple in color. Seeds planted from the crossed ears gave smut reaction for the $F_1$ plants during the 1930 growing season as shown in Table 4.

TABLE 4—*Smut reaction of the $F_1$ plants from a cross of certain selfed strains and a shrunken-waxy tester*

| Description of parents | No. of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|
| | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| (Tassel x sh-wx) $F_1$ ..... | 46 | 1 | 0 | 0 | 0 | 2 | 0 | 6.5 |
| (Base x sh-wx) $F_1$ ....... | 26 | 0 | 0 | 1 | 1 | 4 | 2 | 30.8 |
| (Yel.-resist. x sh-wx) $F_1$ .. | 104 | 2 | 0 | 0 | 0 | 0 | 0 | 1.9 |
| (Wh.-resist. x sh-wx) $F_1$ . | 112 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

Certain of the $F_1$ plants were backcrossed to the shrunken-waxy tester while others were self pollinated. Table 5 gives the color and endosperm classes of the ears obtained. The counts entered in the table are the totals obtained from two or more ears from each backcross or each $F_2$. The combined data obtained from the backcrosses indicate a crossover value of 16.2 percent between the shrunken-waxy loci. This percentage was used in calculating the theoretical numbers in both backcross and $F_2$ populations. P values range from 0.37 to 0.56 for the $F_2$ ears recorded and from 0.18 to 0.37 for the backcross ears. The observed numbers approached the expected class values so closely that the observed deviations might be expected through chance more than twice in five trials (P equalling 0.45) for the $F_2$ ears, and more than twice in seven trials (P equalling 0.28) in the backcross ears. Plantings were made of each of the color and endosperm classes and careful field notes recorded on the smut reaction of each plant during the growing season of 1931. Data obtained are presented in Table 6.

It will be noted that the data are listed to show the parentage of any given material and that comparisons are made only between plants of a given endosperm and genotypic constitution. Only seeds obtained from backcross ears were used in these plantings.

*The order of presenting the data for different linkage groups is that followed in the mimeographed "Summary of Linkage Groups in Maize" prepared by the Department of Plant Breeding of Cornell University, 1929 *(7)*.

TABLE 5—*Color and endosperm condition of backcross and $F_2$ ears obtained from crossing certain selfed strains and a shrunken-waxy tester*

| Description of parents | * | Purple | | | | Red | | | | Yellow | | | | White | | | | $X^2$ and P values |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ShWx | Shwx | shWx | shwx | ShWx | Shwx | shWx | shwx | ShWx | Shwx | shWx | shwx | ShWx | Shwx | shWx | shwx | |
| (Tassel x sh-wx) $F_2$ | O | 118 | 27 | 26 | 49 | | | | | 130 | 14 | 10 | 13 | | | | | $X^2$ = 7.6014 |
| | C | 125 | 20 | 22 | 50 | | | | | 135 | 10 | 7 | 17 | | | | | P = .3709 |
| (Base x sh-wx) $F_2$ | O | 146 | 14 | 24 | 48 | 49 | 8 | 10 | 22 | 169 | 12 | 3 | 12 | 55 | 6 | 1 | 6 | $X^2$ =15.7995 |
| | C | 142 | 22 | 25 | 57 | 47 | 7 | 8 | 19 | 153 | 11 | 8 | 19 | 51 | 4 | 3 | 6 | P = .3959 |
| (Yel.-res. x sh-wx) $F_2$ | O | 235 | 35 | 53 | 84 | | | | | 268 | 17 | 16 | 29 | | | | | $X^2$ = 5.8041 |
| | C | 239 | 37 | 42 | 96 | | | | | 257 | 19 | 14 | 32 | | | | | P = .5633 |
| (Wh.-res. x sh-wx) $F_2$ | O | 74 | 9 | 12 | 21 | 21 | 2 | 3 | 6 | 90 | 10 | 5 | 9 | 27 | 4 | 2 | 5 | $X^2$ =14.8062 |
| | C | 73 | 11 | 13 | 29 | 24 | 4 | 4 | 10 | 79 | 6 | 4 | 10 | 26 | 2 | 1 | 3 | P = .4658 |
| BACKCROSSES | | | | | | | | | | | | | | | | | | |
| (Tassel x sh-wx) x sh-wx B. C. | O | 121 | 30 | 33 | 131 | | | | | | | | | | | | | $X^2$ = 3.2633 |
| | C | 131 | 26 | 26 | 131 | | | | | | | | | | | | | P = .3574 |
| | D | —10 | +4 | +7 | —0 | | | | | | | | | | | | | |
| (Base x sh-wx) x sh-wx B. C. | O | 189 | 29 | 27 | 192 | | | | | | | | | | | | | |
| | C | 183 | 35 | 35 | 183 | | | | | | | | | | | | | $X^2$ = 3.4964 |
| | D | +6 | —6 | —8 | +9 | | | | | | | | | | | | | P = .3270 |
| (Base x sh-wx) x sh-wx B. C. | O | 43 | 4 | 14 | 38 | 47 | 9 | 5 | 53 | | | | | | | | | |
| | C | 44 | 9 | 9 | 14 | 44 | 9 | 9 | 44 | | | | | | | | | $X^2$ =10.2197 |
| | D | +1 | —5 | +5 | —6 | +3 | 0 | —4 | +9 | | | | | | | | | P = .1776 |
| (Yel.-res. x sh-wx) x sh-wx B. C. | O | 66 | 13 | 19 | 66 | | | | | | | | | | | | | |
| | C | 69 | 13 | 13 | 69 | | | | | | | | | | | | | $X^2$ = 5.3776 |
| | D | —3 | 0 | +6 | —3 | | | | | | | | | | | | | P = .1491 |
| (Wh.-res. x sh-wx) x sh-wx B. C. | O | 105 | 17 | 25 | 103 | 100 | 20 | 19 | 116 | | | | | | | | | |
| | C | 106 | 20 | 20 | 106 | 106 | 20 | 20 | 106 | | | | | | | | | $X^2$ = 3.1273 |
| | D | —1 | —3 | +5 | —3 | —6 | 0 | —1 | +10 | | | | | | | | | P = .3730 |

*O=observed ratio; C=calculated ratio; D=difference.

The data have been treated as a two-class frequency distribution, and the significance of the difference in smut reaction is determined by comparison of the probable error of the difference in percentage as obtained from the formula $.6745 \sqrt{\frac{p.q.}{n}}$, in which (n) is the total number of individuals and (p) and (q) are percentages corresponding to the ratios concerned.

None of the endosperm classes obtained from crosses with the West Virginia resistant strains differed significantly from their normal sibs in regard to smut reaction when the above test was applied. The range of values observed varied from 0.5 to 2.9 times the probable error, which is well within the limit of non-significance. On the other hand, both tassel and base West Virginia strains indicate that the endosperm classes tested show significant differences in smut susceptibility from the comparable normal plants of the same family.

TABLE 6—*Smut reaction of backcross plants obtained from planting the different endosperm classes of seed of a cross between certain selfed strains and a shrunken-waxy tester*

| Description of parents | Endosperm condition of plants | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| (Tassel x sh-wx) x sh-wx B. C. | Normal | 38 | 7 | 0 | 1 | 1 | 1 | 0 | 26.3 | |
| | Waxy | 36 | 8 | 1 | 7 | 2 | 2 | 1 | 58.3 | 6.64 |
| (Base x sh-wx) x sh-wx B. C. | Normal | 42 | 14 | 1 | 0 | 1 | 0 | 0 | 38.1 | |
| | Waxy | 33 | 15 | 0 | 3 | 0 | 0 | 1 | 57.6 | 3.86 |
| (Yel.-resist. x sh-wx) x sh-wx B. C. | Normal | 84 | 13 | 0 | 0 | 1 | 0 | 0 | 16.7 | |
| | Waxy | 81 | 3 | 0 | 1 | 3 | 2 | 1 | 12.3 | 1.79 |
| (White-resist. x sh-wx) x sh-wx B. C. | Normal | 112 | 5 | 1 | 1 | 2 | 1 | 0 | 8.9 | |
| | Waxy | 183 | 11 | 1 | 2 | 1 | 2 | 1 | 9.8 | 0.50 |
| (Tassel x sh-wx) x sh-wx B. C. | Normal | 36 | 6 | 0 | 4 | 1 | 1 | 0 | 30.6 | |
| | Shrunken | 38 | 10 | 1 | 4 | 2 | 2 | 1 | 52.6 | 4.37 |
| (Base x sh-wx) x sh-wx B. C. | Normal | 46 | 15 | 1 | 0 | 1 | 0 | 0 | 36.9 | |
| | Shrunken | 29 | 14 | 0 | 3 | 0 | 0 | 1 | 62.1 | 5.25 |
| (Yel.-resist. x sh-wx) x sh-wx B. C. | Normal | 77 | 9 | 0 | 0 | 3 | 0 | 1 | 16.9 | |
| | Shrunken | 88 | 7 | 0 | 1 | 1 | 2 | 0 | 12.5 | 1.85 |
| (White-resist. x sh-wx) x sh-wx B. C. | Normal | 140 | 9 | 2 | 1 | 2 | 2 | 1 | 12.1 | |
| | Shrunken | 157 | 9 | 0 | 2 | 1 | 1 | 0 | 8.3 | 2.56 |
| (Tassel x sh-wx) x sh-wx B. C. | Normal | 26 | 5 | 0 | 0 | 1 | 0 | 0 | 23.1 | |
| | Sh-Waxy | 26 | 8 | 1 | 3 | 2 | 1 | 1 | 61.5 | 6.89 |
| (Base x sh-wx) x sh-wx B. C. | Normal | 37 | 12 | 1 | 0 | 1 | 0 | 0 | 37.8 | |
| | Sh-Waxy | 24 | 12 | 0 | 3 | 0 | 0 | 1 | 66.7 | 5.37 |
| (Yel.-resist. x sh-wx) x sh-wx B. C. | Normal | 51 | 9 | 0 | 0 | 1 | 0 | 0 | 19.6 | |
| | Sh-Waxy | 54 | 3 | 0 | 1 | 0 | 2 | 0 | 11.1 | 2.95 |
| (White-resist. x sh-wx) x sh-wx B. C. | Normal | 70 | 3 | 1 | 1 | 2 | 1 | 0 | 11.4 | |
| | Sh-Waxy | 115 | 7 | 0 | 2 | 1 | 1 | 0 | 9.6 | 0.97 |

It is interesting to note the variations in total amount of smut as shown by a study of the next-to-the-last column in Table 6. Tassel and base are heavily smutted with an average of 42 and 50 percent, respectively, while the total smut of comparable white and yellow-resistant strains shows only about one-fourth as much, with 10 and 15 percent, respectively. Although the base strain does not

seem to maintain its ability to transmit infection to the basal portion of the plants in these populations, it does demonstrate a somewhat greater susceptibility than does the tassel strain. White-resistant backcrosses show on the average about 5 percent less infection than the yellow-resistant backcrosses.

*Linkage Group II*

The genetic tester used to test Linkage Group II was known to be homozygous for AA CC *R-g R-g* prpr, but the results of certain crosses indicated that this tester was also homozygous for (S), a factor which caused the aleurone to be spotted when the endosperm was *rr R*. West Virginia tassel, base, and yellow-resistant strains were pollinated with this tester, and the crossed seed showed spotting, which would be expected if dominant R and S came in from the tester parent.

These crossed seeds were planted. The smut reaction of the $F_1$ plants during the 1930 season is presented in Table 7.

TABLE 7—*Smut reaction of the $F_1$ plants from a cross of certain selfed strains and a spotted golden tester*

| Description of parents | No. of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection |
|---|---|---|---|---|---|---|---|---|
| (Tassel x S-g) $F_1$ ........ | 101 | 19 | 1 | 2 | 3 | 5 | 4 | 33.7 |
| (Base x S-g) $F_1$ ......... | 21 | 0 | 2 | 0 | 2 | 1 | 1 | 28.6 |
| (Yel.-resist. x S-g) $F_1$ .... | 40 | 0 | 1 | 0 | 0 | 0 | 0 | 2.5 |

TABLE 8—*Color and aleurone condition of backcross and $F_2$ ears obtained from crossing certain selfed strains and a spotted-golden tester*

| Description of parents | | Description of aleurone and endosperm: Purple Full | Purple Spotted | Red Full | Red Spotted | Yellow | White | Total |
|---|---|---|---|---|---|---|---|---|
| (Tassel x S.-g) $F_2$ | Observed | 506 | 218 | 172 | 82 | 555 | 190 | 1723 |
| | Calculated | 511 | 216 | 170 | 72 | 565 | 188 | $X^2$=1.6781 |
| | | | | | | | | P = .8856 |
| (Base x S.-g) $F_2$ | Observed | 162 | 62 | 59 | 27 | 190 | 52 | 552 |
| | Calculated | 164 | 69 | 55 | 23 | 181 | 60 | $X^2$=3.2352 |
| | | | | | | | | P = .6646 |
| (Yel.-res. x S.-g) $F_2$ | Observed | 182 | 82 | 78 | 33 | 221 | 63 | 659 |
| | Calculated | 195 | 82 | 65 | 27 | 216 | 72 | $X^2$=6.0407 |
| | | | | | | | | P = .3027 |
| Totals | Observed | 850 | 362 | 309 | 142 | 966 | 305 | $X^2$=5.8586 |
| | Calculated | 870 | 367 | 290 | 122 | 963 | 321 | P = .3217 |
| BACKCROSSES (Tassel x S.-g) x S.-g | B. C. | 90 | 84 | 84 | 92 | $X^2$=1.2257 | | P=.7496 |
| (Base x S.-g) x S.-g | B. C. | 67 | 62 | 66 | 65 | $X^2$= .2154 | | P=.80+ |
| (Yel.-res. x S.-g) x S.-g | B. C. | 349 | 339 | 360 | 370 | $X^2$=1.526 | | P=.6809 |
| Total | Observed | 506 | 485 | 510 | 527 | | | |
| | Calculated | 507 | 507 | 507 | 507 | $X^2$=1.76332 | | P=.6264 |

These data indicate that the $F_1$ plants are intermediate in amount of smut infection. The $F_1$ crosses involving the resistant strain show considerably less smut infection than those of the tassel and base strains. Certain of these $F_1$ plants were backcrossed to

the spotted golden tester while others were self pollinated. Table 8 presents data relative to color and aleurone condition of the backcross and $F_2$ ears produced. The counts recorded in Table 8 represent totals from one or more ears for each cross.

Deviations of observed and calculated numbers were such as might occur by chance about once in three trials, P equalling 0.32 for the $F_2$ ears, while the differences observed in the backcross might occur by chance twice out of three trials. Calculations for the $F_2$ spotted and full-colored endosperm class are based upon a theoretical crossover value of 12½ percent between the R and S loci.

Plantings were made of the different color classes obtained from the backcross ears, and careful notes regarding reaction to smut were recorded for individual plants during the summer of 1931. These data are presented in Table 9.

TABLE 9—*Smut reaction of normal and golden plants obtained from backcross seeds of a cross between certain selfed strains and a spotted-golden tester*

| Description of parents | Plant description | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| (Tassel x S-g) | Normal | 102 | 28 | 0 | 5 | 4 | 6 | 2 | 44.1 | |
| x S-g B. C. | Golden | 81 | 21 | 1 | 1 | 6 | 7 | 0 | 44.4 | 0.09 |
| (Base x S-g) | Normal | 60 | 16 | 1 | 1 | 1 | 5 | 1 | 41.7 | |
| x S-g B. C. | Golden | 45 | 6 | 3 | 3 | 2 | 5 | 1 | 44.4 | 0.67 |
| (Yel.-res. x S-g) | Normal | 57 | 12 | 0 | 3 | 1 | 1 | 0 | 29.8 | |
| x S-g B. C. | Golden | 51 | 8 | 0 | 1 | 3 | 1 | 0 | 25.5 | 1.04 |

TABLE 10—*Smut reaction of the backcross plants obtained from the full-color and spotted-aleurone seeds of a cross between certain selfed strains and a spotted-aleurone tester*

| Description of parents | Aleurone condition of seed | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| (Tassel x S-g) | Full color | 96 | 24 | 1 | 1 | 8 | 4 | 0 | 39.6 | |
| x S-g B. C. | Spotted | 80 | 25 | 0 | 5 | 2 | 4 | 0 | 45.0 | 1.4 |
| (Base x S-g) | Full color | 51 | 8 | 3 | 3 | 2 | 6 | 1 | 45.1 | |
| x S-g B. C. | Spotted | 54 | 14 | 1 | 1 | 1 | 4 | 1 | 40.7 | 0.9 |
| (Yel.-res. x S-g) | Full color | 103 | 16 | 1 | 4 | 4 | 2 | 0 | 26.2 | |
| x S-g B. C. | Spotted | 110 | 23 | 3 | 6 | 1 | 1 | 1 | 31.8 | 1.9 |

It will be seen from the column headed "percent infection" that the amount of smut in the tassel and base strains is nearly double that for the yellow-resistant, but in no cross is there a significant difference between the smut infection of the normal and golden plants when measured by the probable error of the difference.

Table 10 shows no significant differences between the amount of smutting found in the full-color and spotted aleurone classes for the crosses reported. These data are in full agreement with those obtained from the golden and normal plants.

The data presented in Table 11 indicate a linkage between the factors for spotted aleurone and golden plant color. The observed crossover value was 21 percent, which agrees fairly well with the previously reported value of 18.2 percent.

TABLE 11—*Relation between color and condition of aleurone of backcross seeds and the normal and golden plants obtained from these seeds*

| Color of seed | Purple | | Red | |
|---|---|---|---|---|
| | Full color | Spotted | Full color | Spotted |
| Color of plants | 142 golden<br>24 green | 30 golden<br>163 green | 130 golden<br>55 green | 40 golden<br>123 green |

*Linkage Group III*

Linkage Group III is represented by sweet endosperm and tunicate plants. Tassel, base, white-resistant, and yellow-resistant strains were crossed with genetic testers known to be recessive for sweet endosperm. The crossed seed showed no effect of the "su" gene. This is as would be expected, since the West Virginia strains were homozygous starchy. Seeds from the above crosses were planted and notes recorded during the summer of 1930. Certain of these $F_1$ plants were backcrossed to testers recessive for su, while other $F_1$ plants were self-pollinated. Plantings were made of the sweet grains obtained from the backcross ears in the spring of 1931, and comparisons were made with normal plants of comparable genotype. The results are recorded in Table 12.

TABLE 12—*Smut reaction of normal and sweet plants obtained from backcross seeds of a cross between certain selfed strains and a sweet tester*

| Description of parents | Endosperm condition of seed | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Tassel x su) | Normal | 33 | 6 | 0 | 0 | 0 | 4 | 4 | 42.4 | |
| x su B. C. | Sweet | 10 | 3 | 0 | 0 | 0 | 0 | 0 | 30.0 | 1.37 |
| (Base x su) | Normal | 205 | 30 | 7 | 0 | 12 | 18 | 4 | 34.6 | |
| x su B. C. | Sweet | 80 | 2 | 1 | 0 | 3 | 3 | 3 | 15.0 | 7.28 |
| (Yel.-res. x su) | Normal | 77 | 1 | 1 | 1 | 1 | 3 | 1 | 10.4 | |
| x su B. C. | Sweet | 55 | 4 | 1 | 0 | 0 | 0 | 0 | 9.1 | 0.50 |
| (White-res. x su) | Normal | 159 | 13 | 3 | 6 | 5 | 7 | 0 | 21.4 | |
| x su B. C. | Sweet | 96 | 7 | 3 | 1 | 1 | 1 | 0 | 13.5 | 3.36 |

Plants grown from backcross seed involving yellow-resistant and sweet do not show a significant difference between the normal and sweet segregates in regard to smut reaction — a difference of only 0.5 times the probable error being observed. In the cross involving white-resistant and sweet the difference is 3.36 times the probable error, the plants from the sweet seed being the more resistant. This represents a probability of a little over 1 to 38 that the differences may be due to chance. The cross involving the tassel strain is not significant, but that with the base strain is undoubtedly so, with a difference of 7.3 times its probable error. The

tendency is for the sweet plants to show less smut than the normal plants of similar genotypes. These differences are not significant in two crosses, doubtfully significant in a third, and very probably so in a fourth cross.

There are a few data available regarding the tunicate factor, located on the same chromosome with sweet, that may be of value in analyzing the smut relation that exists here. Both white and yellow-resistant strains were crossed with tunicate, which is a dominant plant character. $F_1$ plants produced from this crossed seed were grown in 1930 and self-pollinated. Plants from this seed were grown in 1931 and field notes recorded regarding the reaction to smut. These data are shown in Table 13.

TABLE 13—*Smut reaction of tunicate and normal plants obtained from $F_2$ seeds in a cross between certain selfed strains and a tunicate tester*

| Description of parents | Description of plants | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| (White-resist. x Tu) $F_2$ | Tu | 55 | 8 | 0 | 0 | 0 | 0 | 0 | 14.5 | |
| | tu | 23 | 0 | 1 | 0 | 1 | 0 | 0 | 8.7 | 1.46 |
| (Yel.-resist. x Tu) $F_2$ | Tu | 50 | 10 | 0 | 0 | 0 | 2 | 0 | 24.0 | |
| | tu | 19 | 1 | 0 | 0 | 1 | 1 | 0 | 15.8 | 1.45 |

TABLE 14—*Smut reaction and linkage obtained from the $F_2$ plants in a cross between yellow-resistant and a sweet-tunicate tester*

| Description of parents | Endosperm of seed planted | Starchy: Tunicate Smutted | Starchy: Tunicate Free | Starchy: Normal Smutted | Starchy: Normal Free | Sweet: Tunicate Smutted | Sweet: Tunicate Free | Sweet: Normal Smutted | Sweet: Normal Free |
|---|---|---|---|---|---|---|---|---|---|
| (Yel.-res. x Tu-su) $F_2$ | Appearance and smut infection of $F_2$ plants | 12 | 38 | 3 | 16 | 8 | 12 | .. | 2 |
| | Observed | 50 | | 19 | | 20 | | 2 | |
| | Calculated | 47 | | 21 | | 21 | | 2 | |

There is no indication from these data that the tunicate chromosome is associated with resistance, as one would be led to believe from the evidence from the crosses of sweet factor. The tendency seems to be for the tunicate plants to show greater susceptibility to smut, although this is not marked, being only 1.5 times the probable error. Only one cross is available which includes both tunicate and sweet. These factors came in together in the tester used and were crossed on West Virginia yellow-resistant strain.

The crossed seed was normal in appearance and, when planted, gave rise to $F_1$ plants that were tunicate in appearance. One of these plants on being selfed gave 356 starchy seeds and 106 sweet, as compared to a calculated ratio of 345 starchy to 115 sweet.

In the spring of 1931, 75 of these starch grains and 25 sweet grains were planted and produced plants as shown in Table 14, giving 69 starchy plants and 22 sweet. Notes were recorded on these

plants as indicated in the table, and the segregation into tunicate and normal was determined. The observed classes gave an exceptionally close fit ($X^2 = 0.4296$) to the calculated values when a crossover value of 28.6 percent is assumed between Tu and su loci. The smut reaction of the $F_2$ plants is such as to indicate no linkage relation with either the Tu or su genes.

*Linkage Group IV*

The genetic tester used for Linkage Group IV was known to be recessive for liguleless, a plant character. West Virginia tassel, base, yellow-resistant, and white-resistant strains were pollinated with this tester. The crossed seed was normal in appearance. This was grown in the field during the summer of 1930 and the notes included in Table 15 were recorded for the smut reaction of the $F_1$ plants for the respective crosses.

TABLE 15—*Smut reaction of the $F_1$ plants from the cross of certain selfed strains and a liguleless tester*

| Description of parents | No. of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|
| | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| (Tassel x lg) $F_1$ .......... | 103 | 4 | 0 | 0 | 0 | 24 | 15 | 41.7 |
| (Base x lg) $F_1$ .......... | 102 | 12 | 3 | 1 | 1 | 34 | 8 | 57.8 |
| (Yel.-resist. x lg) $F_1$ ..... | 105 | 0 | 0 | 0 | 0 | 0 | 1 | 1 |
| (White-resist. x lg) $F_1$ ... | 71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

TABLE 16—*Smut reaction of normal and liguleless plants obtained from backcross seeds in a cross between certain selfed strains and a liguleless tester*

| Description of parents | Description of plants | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Tassel x lg) $F_2$ | Lg | 70 | 17 | 2 | 2 | 3 | 0 | 4 | 40.0 | |
| | lg | 16 | 3 | 1 | 0 | 0 | 0 | 0 | 25.0 | 2.05 |
| (Tassel x lg) x lg B. C. | Lg | 43 | 9 | 0 | 0 | 0 | 4 | 4 | 39.5 | |
| | lg | 62 | 6 | 0 | 1 | 0 | 11 | 4 | 35.5 | 0.98 |
| (Base x lg) x lg B. C. | Lg | 50 | 5 | 1 | 0 | 2 | 9 | 4 | 42.0 | |
| | lg | 49 | 2 | 3 | 1 | 0 | 15 | 3 | 49.0 | 1.49 |
| (Yel.-resist. x lg) x lg B. C. | Lg | 67 | 1 | 0 | 0 | 2 | 5 | 1 | 13.4 | |
| | lg | 94 | 2 | 0 | 0 | 3 | 30 | 8 | 45.7 | 11.51 |
| (White-resist. x lg) x lg B. C. | Lg | 58 | 5 | 1 | 0 | 0 | 3 | 0 | 15.5 | |
| | lg | 49 | 5 | 0 | 1 | 0 | 4 | 1 | 22.5 | 2.18 |

The $F_1$ plants from the tassel and base crosses show a high percentage of smut. This would suggest that the liguleless tester has contributed susceptible genes that are similar to those borne by the West Virginia susceptible parent strains. If this is the correct assumption, the factors for susceptibility contributed by the liguleless tester behave as recessives when they are associated with either of the West Virginia resistant strains.

Certain of the $F_1$ plants from each cross were backcrossed to the liguleless tester, and the seed thus obtained was planted in 1931. Table 16 presents data which show the comparison of normal and liguleless plants in regard to smut reaction.

Neither backcrosses involving tassel or base strains gave a significant difference in the amount of smut observed in the normal and liguleless segregates — the difference observed being 0.98 and 1.49 times the probable error for these strains. In crosses with white and yellow-resistant strains, however, differences of 2.2 and 11.5 times their probable errors were observed. This would suggest that there is a significant relation between the smutted plants in the normal and liguleless classes and that the liguleless character is associated with susceptibility. Just what this relation is, whether morphological or physiological, is undetermined at present. It is hoped to study this chromosome more completely by building up testers of known smut reaction in order to determine any linkage relations that may exist.

TABLE 17—*Smut reaction of yellow and non-yellow plants obtained from the backcross seed of certain selfed strains and a yellow-endosperm tester*

| Description of parents | Description of endosperm of seed | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Tassel x y) x y B. C. | Yellow | 164 | 32 | 2 | 0 | 12 | 5 | 3 | 32.9 | |
| | Non-yellow | 70 | 13 | 3 | 0 | 5 | 5 | 0 | 37.1 | 1.08 |
| (Base x y) x y B. C. | Yellow | 114 | 15 | 8 | 1 | 4 | 8 | 3 | 34.2 | |
| | Non-yellow | 70 | 7 | 0 | 0 | 8 | 7 | 0 | 31.4 | 0.75 |
| (Yel.-res. x y) x y B. C. | Yellow | 77 | 3 | 0 | 1 | 2 | 0 | 1 | 9.1 | |
| | Non-yellow | 79 | 2 | 2 | 1 | 2 | 1 | 0 | 10.1 | 0.45 |
| (White-res. x y) x y B. C. | Yellow | 37 | 0 | 3 | 0 | 0 | 0 | 0 | | |
| | Non-yellow | 28 | 0 | 0 | 0 | 0 | 0 | 0 | | |

*Linkage Group V*

Data are brought together in Table 17 to represent the difference between yellow and non-yellow endosperm. All of the West Virginia strains except the white-resistant carried yellow endosperm. These data represent plants grown from backcross seed that was either yellow or non-yellow, as shown by examination of the seed prior to planting in the spring of 1931. It is apparent from the differences shown in the last column of this table that there is no difference in the yellow and non-yellow endosperm plants in regard to reaction to smut.

*Linkage Group VI*

The genetic tester used for Linkage Group VI was known to be recessive for brachytic and fine stripe. Both are plant characters, the former causing a marked shortening of the internodes and giving a characteristic telescoped appearance to the plant, while the latter causes the leaves to be striped. The latter character was not easily classified in the segregating material, but the brachytic character was very sharply defined.

West Virginia base, yellow, and white-resistant strains were pollinated with the brachytic fine-stripe pollen, and plants from the

TABLE 18—*Smut reaction of the $F_1$ plants from a cross of certain selfed strains and a brachytic fine-stripe tester*

| Description of parents | No. of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|
| | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| (Tassel x br-f) $F_1$ ........ | 99 | 7 | 0 | 1 | 17 | 21 | 1 | 47.5 |
| (Base x br-f) $F_1$ ........ | 101 | 6 | 1 | 0 | 3 | 20 | 14 | 42.3 |
| (Yel.-res. x br-f) $F_1$ ...... | 51 | 0 | 0 | 0 | 2 | 0 | 2 | 7.8 |
| (White-res. x br-f) $F_1$ .... | 90 | 1 | 1 | 0 | 0 | 0 | 0 | 2.2 |

seed thus produced were grown during the 1930 season. Smut reaction of these $F_1$ plants is recorded in Table 18.

These data are somewhat similar to other $F_1$ data obtained during 1930. The resistant strains seem to demonstrate their resistance in these crosses; the white-resistant parent apparently is much more resistant than the yellow-resistant.

Certain of these $F_1$ plants were backcrossed with pollen from the brachytic fine stripe tester, while other $F_1$ plants were self-pollinated. These backcross seeds were planted in 1931, and the reaction of the base and two resistant families to smut infection during 1931 is recorded in Table 19.

The results from the data obtained from backcrosses in all three families indicate that there is more smut infection in the brachytic than in the normal plants within the same family. In the base strain this amounts to 2.4 times the probable error, while in the yellow and white-resistant strains the difference is 3.1 and 5 times the probable error, respectively.

TABLE 19—*Smut reaction of normal and brachytic plants obtained from backcross seed in a cross between certain selfed strains and a brachytic fine-stripe tester*

| Description of parents | Description of plants | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Base x br-f) | Br | 95 | 4 | 0 | 2 | 1 | 27 | 7 | 43.2 | |
| x br-f B. C. | br | 117 | 6 | 0 | 3 | 36 | 10 | 5 | 51.3 | 2.36 |
| (Yel.-res. x br-f) | Br | 137 | 16 | 1 | 2 | 2 | 10 | 1 | 23.4 | |
| x br-f B. C. | br | 149 | 6 | 1 | 1 | 15 | 19 | 4 | 30.9 | 3.07 |
| (Wh.-res. x br-f) | Br | 42 | 2 | 0 | 0 | 1 | 1 | 1 | 11.9 | |
| x br-f B. C. | br | 59 | 6 | 0 | 0 | 9 | 2 | 0 | 28.8 | 5.0 |

These two latter cases are probably significant and since they are based upon data obtained from resistant West Virginia strains they indicate a relationship between smut susceptibility and the brachytic character.

Since the tester used carried both brachytic and fine stripe recessive characters it was possible to identify the crossover classes from the backcross populations. From a total backcross population of 1167 plants 529 were normal, 39 normal fine-stripe, 37 brachytic normal-leaf, and 562 were brachytic fine-stripe. This gives a calculated crossover value of 6.5 percent between the br and f loci. This is somewhat higher than the value of 5.4 percent reported by

previous workers *(7)*. Altogether 218 $F_2$ plants were grown that were observed to segregate as follows:

| | Br F | Br f | br F | br f |
|---|---|---|---|---|
| Observed | 170 | 6 | 4 | 38 |
| Calculated | 155 | 6 | 6 | 47 |

$X^2 = 3.8417$

The P value obtained is .282, which indicates a good fit to expected ratios. Although there were only 39 crossover plants that were normal fine-stripe and 37 brachytic non-striped, there is nearly twice as much smut in the latter as in the former group. That portion of the chromosome can be identified which originated with the West Virginia strains. This fact suggests that factors for smut susceptibility are more closely associated with the brachytic locus than with the locus for fine stripe. It is unfortunate that the numbers are limited and that all of the crossover plants could not have arisen from West Virginia resistant strains.

*Linkage Group VII*

The genetic tester used for Linkage Group VII was ramosa — a character which affects plant and ear; the tassel usually has but one main spike with a tendency for the secondary spikes to be greatly branched. The ear shoots at first appear normal but early show a branching tendency which causes the husks to rupture, leaving the bulk of the developing ear exposed.

West Virginia base, yellow, and white-resistant strains were crossed with pollen from the ramosa tester. This seed was planted in the spring of 1930 and some of the $F_1$ plants were smutted as shown in Table 20. These data indicate a rather heavy infection for West Virginia tassel and base strains but show marked resistance for the two resistant strains.

TABLE 20—*Smut reaction of the $F_1$ plants obtained from a cross between certain selfed strains and a Ramosa tester*

| Description of parents | No. of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|
| | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| (Tassel x ra) $F_1$ .......... | 47 | 1 | 0 | 0 | 6 | 11 | 2 | 42.6 |
| (Base x ra) $F_1$ .......... | 99 | 0 | 0 | 0 | 6 | 18 | 12 | 36.4 |
| (Yel.-res. x ra) $F_1$ ....... | 88 | 0 | 0 | 1 | 2 | 2 | 0 | 5.7 |
| (White-res. x ra) $F_1$ ...... | 78 | 0 | 0 | 0 | 1 | 1 | 0 | 2.6 |

Certain of these $F_1$ plants were backcrossed with pollen from the ramosa tester, and plants were grown from this seed during the 1931 season. Data showing the smut reaction of normal and ramosa plants coming from each backcross are presented in Table 21.

Practically all of the smut found on ramosa plants was observed on the ears. The percentage of smutted ramosa plants is nearly the same whether the plant comes from a base or a resistant strain. When the relation between the normal and ramosa plants is measured by the criterion used in previous crosses, this difference is found to be highly significant.

These data seem to indicate that gross morphology plays a very important role in the smut behavior of plants and that, in this instance at least, the morphology of the plant has overcome, to a great extent, any physiological resistance which might have been present.

TABLE 21—*Smut reaction of normal and ramosa plants obtained from the backcross seed in a cross between certain selfed strains and a ramosa tester*

| Description of parents | Description of plants | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection | P. E. / Diff. |
|---|---|---|---|---|---|---|---|---|---|---|
| (Base x ra) | Ra | 56 | 4 | 6 | 3 | 8 | 1 | 0 | 39.3 | |
| x ra B. C. | ra | 52 | 3 | 0 | 0 | 46 | 0 | 0 | 94.2 | 12.5 |
| (Yel.-res. x ra) | Ra | 55 | 6 | 0 | 0 | 3 | 1 | 0 | 18.2 | |
| x ra B. C. | ra | 47 | 0 | 0 | 0 | 43 | 1 | 0 | 93.6 | 21.5 |
| (Wh.-res. x ra) | Ra | 62 | 2 | 0 | 0 | 0 | 0 | 2 | 6.5 | |
| x ra B. C. | ra | 39 | 1 | 0 | 0 | 29 | 0 | 1 | 82.1 | 35.8 |

*Linkage Group VIII*

Plants representing Linkage Group VIII came from three sources. The smut reaction of the plants produced from seeds with purple and red aleurone previously reported in Tables 5, 6, 8, and 10 differ with respect to the "Pr" gene and can be used to represent this linkage group. No significant differences were observed in the smut reaction of the normal plants coming from purple and red aleurone seeds; therefore the data are not repeated in this section.

Brevis-like *(25)* plants were found as segregates in the $F_2$ and certain backcrosses with two of the genetic testers (although these testers were not known to carry the brevis factor at the time they were used as pollinators). The segregating plants give some information in regard to the smut reaction of this character. The tester used to represent Linkage Group VIII was virescent #2. West Virginia tassel, yellow, and white-resistant strains were pollinated by virescent #2 pollen. The crossed seed was grown during 1930 and the smut reaction of the $F_1$ plants is recorded in Table 22.

TABLE 22—*Smut reaction of the $F_1$ plants obtained from a cross between certain selfed strains and a virescent No. 2 tester*

| Description of parents | No. of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percent infection |
|---|---|---|---|---|---|---|---|---|
| (Tassel x $V^2$) $F_1$ .......... | 49 | 6 | 1 | 0 | 0 | 3 | 0 | 20.4 |
| (Yel.-res. x $V^2$) $F_1$ ........ | 55 | 0 | 0 | 0 | 0 | 3 | 1 | 7.3 |
| (Wh.-res. x $V^2$) $F_1$ ....... | 105 | 1 | 1 | 0 | 0 | 0 | 0 | 1.9 |

The amount of smut obtained from the $F_1$ plants of the cross between West Virginia strains and the virescent #2 tester is somewhat less than that obtained for most $F_1$ plants. The West Virginia resistant strains seem to show about their usual amount of resistance. Certain of the $F_1$ plants grown in 1930 were back-

crossed with pollen from the virescent #2 tester, and the field notes taken of the plants grown from this backcross seed are recorded in Table 23.

It is of particular interest to note here that there is apparently a significant difference between the normal and virescent #2 plants in regard to amount of smut infection: the virescent #2 plants show less smut in each of the three families. This difference is 7.1 times its probable error in the cross with West Virginia tassel strain and 5 times its probable error when both resistant families are considered, but only 2 times the probable error with the white-resistant strain alone.

TABLE 23—*Smut reaction of normal and virescent plants obtained from backcross seed of a cross between certain selfed strains and a virescent No. 2 tester*

| Description of parents | Description of plants | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Tassel x $V^2$) x $V^2$ B. C. | Normal | 77 | 30 | 1 | 0 | 3 | 4 | 3 | 53.2 | |
| | Virescent | 24 | 2 | 1 | 0 | 1 | 0 | 0 | 16.7 | 7.1 |
| (Yel.-res. x $V^2$) x $V^2$ B. C. | Normal | 80 | 1 | 1 | 0 | 3 | 2 | 0 | 8.7 | |
| | Virescent | 20 | 0 | 0 | 0 | 0 | 0 | 0 | 0.0 | 5.0 |
| (Wh.-res. x $V^2$) x $V^2$ B. C. | Normal | 104 | 3 | 4 | 0 | 0 | 0 | 0 | 3.6 | |
| | Virescent | 104 | 1 | 0 | 0 | 1 | 0 | 0 | 1.9 | 1.9 |

TABLE 24—*Smut reaction of normal and brevis plants obtained from $F_2$ and backcross seeds of a cross between certain selfed strains and a brevis tester*

| Description of parents | Description of plants | Number of plants | Place of infection | | | | | | Percent infection | Diff. / P. E. |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | | |
| (Tassel x bv) $F_2$ | Normal | 69 | 17 | 2 | 2 | 3 | 0 | 4 | 40.6 | |
| | Brevis | 22 | 2 | 0 | 0 | 1 | 0 | 4 | 33.3 | 1.1 |
| (Base x bv) x bv B. C. | Normal | 28 | 4 | 0 | 1 | 6 | 3 | 2 | 57.1 | |
| | Brevis | 15 | 4 | 1 | 0 | 7 | 0 | 0 | 80.0 | 3.3 |
| (Yel.-res. x bv) x bv B. C. | Normal | 133 | 10 | 9 | 1 | 12 | 4 | 4 | 30.1 | |
| | Brevis | 27 | 1 | 3 | 0 | 11 | 6 | 0 | 74.1 | 7.7 |

During the late summer of 1931 certain families were observed to be segregating for brevis-like plants. The factor for brevis apparently was carried in the heterozygous condition in both the liguleless and crinkly dwarf tester. The smut reactions of the brevis and normal plants which appeared in crosses with West Virginia tassel, base, and yellow-resistant strains are shown in Table 24.

Although the number of plants is not large, the difference in smut reaction of the normal and brevis plants clearly indicates the greater susceptibility of the brevis plants. It is particularly interesting to note that of the three types of plants that represent this linkage group, none shows similar smut reaction. Those plants that differ only by the "Pr" gene, that determines aleurone color, show no relation to smut, while the virescent #2 plants are resistant and the brevis plants show an equally striking smut susceptibility.

*Linkage Group IX*

The genetic tester used for Linkage Group IX was represented by crinkly, dwarf$_1$, and tassel seed #4. Recent evidence obtained by Brink *(2)*, Emerson, Hofmeyr *(15)*, and by McClintock *(27)* indicates that the $d_1$-$pg_2$ group and the A-$ts^4$ group are in reality all linked, and these are discussed as representing Linkage Group IX. The genetic tester used to represent this group was known to be segregating for crinkly, dwarf$_1$, and tassel seed #4. West Virginia tassel, base, yellow, and white-resistant strains were crossed with the Group IX tester, and the reaction of the $F_1$ plants obtained is presented in Table 25.

TABLE 25—*Smut reaction of the $F_1$ plants obtained from a cross between certain selfed strains and a crinkly-dwarf$_1$-tassel seed No. 4 tester*

| Description of parents | No. of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|
| | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| (Tassel x cr-$d_1$-$ts^4$) $F_1$ .... | 51 | 16 | 0 | 0 | 2 | 9 | 0 | 52.9 |
| (Base x cr-$d_1$-$ts^4$) $F_1$ ...... | 65 | 3 | 0 | 0 | 2 | 8 | 5 | 27.7 |
| (Yel.-res. x cr-$d_1$-$ts^4$) $F_1$ .. | 91 | 3 | 3 | 0 | 5 | 2 | 0 | 14.3 |
| (White-res. x cr-$d_1$-$ts^4$) $F_1$ | 29 | 0 | 1 | 0 | 0 | 0 | 0 | 3.4 |

Certain $F_1$ plants were backcrossed to the crinkly-dwarf$_1$-tassel seed #4 tester while others were self-pollinated. The smut reaction of these backcrosses and $F_2$ plants is given in Table 26.

TABLE 26—*Smut reaction of normal, crinkly, dwarf$_1$, and tassel seed No. 4 plants obtained from the $F_2$ and backcross seed of a cross between certain selfed strains and a crinkly-dwarf$_1$-tassel seed No. 4 tester*

| Description of parents | Phenotype of B. C. and $F_2$ plants | Number of plants | Place of infection | | | | | | Percent infection |
|---|---|---|---|---|---|---|---|---|---|
| | | | Tassel | Neck | Leaf | Ear | Below ear | Base | |
| Base x cr-$d_1$-$ts^4$ $F_2$ | Cr D $TS^4$ | 47 | 4 | 3 | 0 | 4 | 7 | 2 | 42.6 |
| | Cr D $ts^4$ | 26 | 24 | 0 | 0 | 1 | 0 | 1 | 100.0 |
| | cr D $TS^4$ | 5 | 1 | 0 | 0 | 0 | 2 | 1 | 80.0 |
| | cr D $ts^4$ | 6 | 6 | 0 | 0 | 0 | 0 | 0 | 100.0 |
| | cr d $Ts^4$ | 9 | 0 | 0 | 1 | 2 | 0 | 0 | 33.3 |
| | cr d $ts^4$ | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 100.0 |
| Base x cr-$d_1$-$ts^4$ B. C. | $TS^4$ | 79 | 39 | 2 | 1 | 2 | 8 | 4 | 70.9 |
| | $ts^4$ | 28 | 27 | 0 | 0 | 0 | 0 | 0 | 96.5 |
| | Cr $TS^4$ | 75 | 9 | 1 | 0 | 0 | 10 | 22 | 56.0 |
| | Cr $ts^4$ | 6 | 5 | 0 | 0 | 0 | 1 | 0 | 100.0 |
| | cr $TS^4$ | 10 | 1 | 0 | 0 | 1 | 3 | 3 | 80.0 |
| | cr $ts^4$ | 15 | 13 | 0 | 0 | 0 | 0 | 0 | 86.7 |
| Base x cr-$d_1$-$ts^4$ B. C. | Cr D $TS^4$ | 28 | 4 | 0 | 1 | 6 | 3 | 2 | 57.1 |
| | Cr D $ts^4$ | 11 | 7 | 0 | 0 | 4 | 0 | 0 | 100.0 |
| | cr d $TS^4$ | 30 | 4 | 2 | 0 | 10 | 3 | 0 | 63.3 |
| | cr d $ts^4$ | 6 | 4 | 2 | 0 | 0 | 0 | 0 | 100.0 |
| Yellow-resist. x cr-$d_1$-$ts^4$ B. C. | Cr D | 102 | 10 | 9 | 0 | 11 | 3 | 3 | 35.3 |
| | cr D | 5 | 0 | 0 | 0 | 2 | 0 | 0 | 40.0 |
| | Cr d | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 00.0 |
| | cr d | 71 | 1 | 3 | 1 | 13 | 4 | 1 | 32.4 |
| White-resist. x cr-$d_1$,$ts^4$ $F_2$ | Cr D $TS^4$ | 59 | 11 | 0 | 0 | 0 | 0 | 1 | 20.3 |
| | Cr D $ts^4$ | 10 | 9 | 0 | 0 | 0 | 0 | 0 | 90.0 |
| | cr D $TS^4$ | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 00.0 |
| | cr D $ts^4$ | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 00.0 |
| | cr d $TS^4$ | 12 | 0 | 0 | 0 | 0 | 0 | 0 | 00.0 |
| | cr d $ts^4$ | 17 | 6 | 0 | 0 | 0 | 0 | 0 | 35.3 |

The number of plants listed under the different phenotypes of the backcross and $F_2$ segregating families is not large. The nature of the tester used is indicated by the type of segregation shown in the column headed "phenotypes of backcross and $F_2$ plants." For example, each of the three backcross ears grown from the West Virginia base x crinkly-dwarf$_1$-tassel seed #4 tester shows a different kind of segregation. No attempt has been made to fit the numbers of observed plants to calculated ratios; it is felt that the discrepancies can be accounted for by the differential viability of the tassel seed #4 and dwarf$_1$ plants.

The amount of smut observed for the different segregating types is very high. This is to be expected in those types where West Virginia base strain was used as one of the parents. It is of particular interest to see that the tassel seed #4 plants gave practically 100 percent infection and that nearly all of this smut was in the tassel portion of the infected plants. Where the West Virginia resistant strains are used as parents the smut is still high, but materially less than with the base strain as a parent.

*Summary of Linkage Groups*

Table 27 is a summary which shows the place and percentage of smut infection for all plants grown in 1931. These plants in some cases represent normal and segregating types from crosses involving West Virginia strains other than those reported previously. The chief purpose for presenting the data in this form is to bring together individuals in order to show the tendency of smut reaction for the different linkage groups.

The data have been grouped without regard to West Virginia parentage. Plants represented by Linkage Groups IV, VI, VII, IX, and the brevis plants of Group VIII show a greater percentage of smut in the segregates than in the normal plants within the same family.

If the above inference is correct: i. e., if these testers are concerned in the transfer of smut-susceptible genes as seems to be indicated by a study of the backcross and $F_2$ progeny involving these testers, it should be possible to detect differences in the reaction of the $F_1$ plants when the different testers are crossed with West Virginia strains. Table 28 shows the average smut infection of the $F_1$ plants from crosses between Linkage Groups I, II, and VIII with certain West Virginia strains, as compared with the $F_1$ plants from Linkage Groups IV, VI, VII, and IX with the same West Virginia strains. It is apparent that there is more smut produced on the $F_1$ plants with certain testers than with others. The reasons for this smut reaction of $F_1$ plants and its true significance are not apparent at the present time. Such results might be expected if the West Virginia, tassel, and base strains carry the same factors for susceptibility as those borne by the linkage testers. These susceptible factors could very easily be common to the testers in question and

may or may not be a function of the particular characteristic of the tester. The fact that neither of the West Virginia resistant strains shows this smut relation in $F_1$ plants would suggest that they carry factors that behave as dominants to those contributed by the genetic tester.

TABLE 27—*Summary of all plants, showing normals vs. segregates, with places and percentage of smut indicated*

| Group | Name | Number of plants | Place of infection: Tassel | Neck | Leaf | Ear | Below ear | Base | Percentage of smut infection |
|---|---|---|---|---|---|---|---|---|---|
| I | Normal | 525 | 69 | 9 | 7 | 10 | 5 | 3 | 19.6 |
| | Shrunken | 496 | 68 | 3 | 11 | 7 | 10 | 3 | 20.6 |
| | Normal | 489 | 74 | 7 | 5 | 8 | 7 | 0 | 20.7 |
| | Waxy | 428 | 61 | 5 | 13 | 9 | 8 | 6 | 23.8 |
| | Normal | 347 | 55 | 7 | 3 | 7 | 3 | 0 | 21.6 |
| | Shrunken-waxy | 354 | 49 | 3 | 9 | 6 | 6 | 3 | 21.5 |
| II | Normal | 447 | 77 | 6 | 14 | 18 | 25 | 6 | 32.7 |
| | Golden | 313 | 63 | 8 | 8 | 16 | 16 | 3 | 36.4 |
| | Full color | 435 | 83 | 7 | 13 | 19 | 18 | 3 | 32.9 |
| | Spotted | 427 | 75 | 9 | 15 | 17 | 23 | 7 | 34.2 |
| III | Normal | 71 | 3 | 1 | 0 | 1 | 1 | 1 | 9.8 |
| | Tunicate | 185 | 29 | 0 | 0 | 1 | 4 | 0 | 18.4 |
| | Normal | 1129 | 93 | 29 | 17 | 34 | 65 | 24 | 23.2 |
| | Sweet | 512 | 27 | 7 | 6 | 7 | 14 | 5 | 12.9 |
| IV | Normal | 629 | 55 | 12 | 4 | 16 | 45 | 21 | 24.3 |
| | Liguleless | 443 | 31 | 5 | 3 | 8 | 89 | 28 | 37.0 |
| V | Yellow | 1229 | 139 | 19 | 23 | 35 | 69 | 26 | 25.3 |
| | Non-yellow | 910 | 116 | 22 | 18 | 33 | 37 | 19 | 26.9 |
| | Purple | 432 | 37 | 5 | 0 | 16 | 26 | 14 | 22.7 |
| | Sunred | 333 | 36 | 4 | 5 | 12 | 9 | 12 | 23.4 |
| | Brown | 97 | 9 | 1 | 0 | 3 | 5 | 1 | 19.6 |
| | Green | 122 | 12 | 0 | 3 | 2 | 6 | 3 | 21.3 |
| VI | Normal | 692 | 57 | 7 | 9 | 22 | 69 | 26 | 27.4 |
| | Brachytic | 639 | 36 | 3 | 6 | 130 | 76 | 11 | 41.0 |
| VII | Normal | 403 | 23 | 14 | 6 | 18 | 9 | 4 | 18.4 |
| | Ramosa | 352 | 7 | 0 | 0 | 273 | 5 | 2 | 81.5 |
| VIII | Normal | 499 | 62 | 9 | 2 | 8 | 11 | 11 | 20.6 |
| | Virescent No. 2 | 192 | 5 | 2 | 0 | 2 | 1 | 0 | 5.2 |
| | Normal | 404 | 37 | 18 | 4 | 31 | 23 | 12 | 30.9 |
| | Brevis | 85 | 2 | 7 | 0 | 26 | 13 | 5 | 62.3 |
| IX | Normal | 536 | 37 | 48 | 2 | 47 | 36 | 15 | 34.5 |
| | $Dwarf_1$ | 287 | 10 | 8 | 2 | 66 | 25 | 2 | 39.4 |
| | Normal | 361 | 73 | 8 | 3 | 25 | 36 | 35 | 49.9 |
| | Tassel seed No. 4 | 133 | 108 | 2 | 0 | 5 | 1 | 1 | 88.0 |

TABLE 28—*Smut reaction of $F_1$ plants from crosses between certain West Virginia strains and known genetic testers*

| Strain | Percent smut of Linkage Groups: I - II - VIII | IV - VI - VII - IX |
|---|---|---|
| Tassel | 24.0 | 45.7 |
| Base | 29.8 | 42.4 |
| Yellow-resistant | 3.5 | 6.9 |
| White-resistant | 0.9 | 1.9 |

Several cases have been presented where the data indicated that gross morphology of the plant was the determining cause of smut reaction. It is possible that the morphology of the plant plays an important role in those selfed strains where resistance and susceptibility can be demonstrated but where it has not been possible

to observe changes in form that will account for the reaction. The $F_1$ plants resulting from crosses with linkage testers are of particular interest in this connection, since they are all normal in appearance. It is therefore not so probable that gross morphology of the plant is playing such an important role in determining smut reaction.

The work of Immer *(17)* at Minnesota suggests a linkage with the "B" and "P" groups which corresponds with Groups IV and VI of this report, but he failed to obtain indication of linkage in "Bn" and "A" groups, which correspond to groups VII and IX of this report. Since Linkage Groups VII and IX were the ones showing the most distinct relationship between morphology and smut reaction, it would appear that choice of a linkage tester with respect to morphological characters is very important in such studies.

This emphasizes the importance of knowing something about the smut reaction of the linkage tester used. Work is now under way at the West Virginia Experiment Station looking toward the synthesis of linkage testers that will give definite smut reaction similar to the selfed lines reviewed earlier in this paper. When these linkage testers are obtained it is hoped that crucial tests for smut inheritance can be made.

## DISCUSSION

Certain selfed strains of maize were grown under smut-epiphytotic conditions and careful notes were recorded on the smut reaction of individual plants within each selfed line. Certain lines of maize were obtained which showed sharp differences in regard to place and amount of smut infection. Crosses between these selfed lines showed clearly that reaction to smut is a strain peculiarity that can be transmitted from parent to offspring.

Segregation observed in the backcross and $F_2$ generations indicated that a complex condition exists and that many factors are concerned in inheritance.

Crosses between West Virginia selfed lines and certain linkage testers indicate that at least four testers show a linkage relation with smut susceptibility. The $F_1$ plants corroborate the conclusions in regard to the above susceptible groups and in a manner to minimize the peculiar morphology of the plant.

Gross morphology no doubt plays a very important role in the reaction of smut. The fourth linkage group is represented by the liguleless character, which produces a plant that does not have the leaf sheath tightly clasped about the main stalk. Most of the smut reported for these plants was found in the lower regions of the plant. This might follow if the smut spore were to fall on the leaf and be washed back of the leaf sheath, where conditions are favorable for germination and infection.

Linkage Group VI is represented by the brachytic factor, which causes a marked dwarfing or shortening of plant internodes. When the ear shoots appear the leaf sheaths are ruptured, and an excellent

focal point for infection of the inner meristematic tissue is presented. Most of the infected brachytic plants observed were found to be infected on the ear and below the ear.

Linkage Group VII is represented by "ramosa". The most pronounced feature is the excessive branching of the ear, which soon causes the protecting husks to rupture and expose the young spikelets of the developing ear. It was observed that over 95 percent of the "ramosa" plants were smutted in the ear. The "ramosa" plants obtained from the yellow and white-resistant West Virginia strains showed 93.6 and 82.1 percent infection, respectively, which would indicate, in this case at least, that the morphology of the plant had a greater influence on smut reaction than any resistance that came in from their respective West Virginia parents.

Linkage Group VIII was represented by the virescent #2 tester as well as certain brevis-like plants that were observed to segregate from other genetic testers.

The $F_1$ plants as well as the backcross and $F_2$ plants from the virescent #2 tester showed marked resistance to smut. These virescent #2 plants were observed to be late and weaker than their normal sibs. The fact that they were late might have let them escape smut infection; or it is possible that the stunted growth also brought about a physiological condition within the plant that was not favorable to smut infection.

"Brevis" is a recessive plant character that causes decided shortening of the internodes of the plant. The character resembles "brachytic" in this respect, but the shortening of the internodes is limited to a portion of the plant. The result generally produces a semi-dwarf condition with a characteristic bunching of the leaf blades. In most cases there is a marked twisting of the culm that accompanies the shortening of the internodes. This tendency for the culms to twist causes the leaf sheaths to split and expose the young tissue of the plant, usually in the vicinity of the ear shoot. This condition produces a favorable point of entrance for the smut spores and may be one reason why the "brevis" plants were found to show such high percentages of smut when compared to their normal sibs.

Linkage Group IX is represented by the crinkly-dwarf$_1$-tassel seed #4 tester. "Crinkly-dwarf$_1$" and (A-ts$^4$) were formerly considered as separate groups, but recent work by Brink, by Emerson, by Hofmeyr, and by McClintock indicates that the old groups IX and X are linked; therefore they are discussed together.

The genetic material used for a tester was segregating for crinkly, dwarf$_1$, and tassel seed #4. The latter character produces seed in the tassel and will not produce an ear shoot unless the tassel is removed. The $F_1$ plants were obtained by crossing the West Virginia strains on a crinkly-dwarf$_1$ tassel seed #4 plant. Backcrosses were made by using pollen from crinkly-dwarf plants which, in some of the cases reported, were also heterozygous for tassel

seed #4. These data are all presented in Table 26 and need not be discussed further here. The most interesting feature about the data is the behavior of the tassel seed #4 plants in regard to smut reaction. These plants approach 100 percent infection when the West Virginia base strain is used as one parent. Here again is an excellent place to show the effect of a favorable morpho'ogy upon the amount of smut infection produced. Tassel seed #4 normally produces a mass of silks and seed in the tassel. This mass is exposed soon after emergence and offers an excellent place for infection to occur. Although the amount of smut infection is high when the West Virginia resistant strains are used as the parents, there is a decided decrease in the amount of smut from that observed for the West Virginia base strain.

The data indicate that reaction to smut is probably conditioned by two sets of factors. One controls the physiological behavior of the plant; the other is concerned with its morphological characteristics.

The presence of factors governing physiological resistance is well illustrated in crosses involving the West Virginia yellow and white-resistant strains. These strains exert their influence on the smut reaction of progeny even where the morphology is such that the plants should smut heavily.

There are also cases where the gross morphology apparently obscures any physiological resistance that is present as seen in the cross with "ramosa".

The $F_3$ generation of the cross between yellow and white-resistant strains has given a population of highly-resistant plants which show considerable promise as foundation stock for further breeding work. The present indications are that a resistant strain with highly desirable agronomic characters can be obtained.

## SUMMARY

Selfed lines of maize that show sharp differences in amount and place of smut infection have been isolated.

The $F_1$ plants from crosses between certain selfed lines of maize that differ in amount of smut infection show an amount of smut intermediate to that of the parent lines.

The tendency of the offspring to resemble the parent lines in regard to place of infection is less clearly defined than their reaction to amount of smut.

Direct and reciprocal crosses in general gave similar results.

Results of crosses between certain selfed lines and representatives of nine linkage groups indicate that at least Groups IV, VI, VII, and IX are associated with smut susceptibility. It is shown that each of the above groups was represented by plants where gross morphology might play an important role in the reaction of a plant to smut.

The $F_3$ progeny resulting from the intercrosses of the most resistant selfed lines indicate that it will be possible to isolate desirable agronomic types that are highly smut-resistant.

It is concluded that in so far as the host is concerned two sets of genetic factors seem to control the reaction of any particular strain to smut. One group of factors is concerned primarily in the control of physiological behavior and the second is concerned with the morphology of the plant. It is felt that by more complete testing of the linkage groups, certain genetic factors not readily identified with morphological differences may be found that will show linkage with factors controlling physiological conditions.

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